Method Development and Validation for Simultaneous Estimation of Metform in Hydrochloride and Nateglinide by Rp-Hplc Method in Its Pure and Tablet Dosage Form

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Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin Hydrochloride (MET) and Nateglinide (NAT) in Tablet dosage form. Chromatogram was run through Symmetry C18 (150 x 4.6 mm, 3.5µ) Mobile phase containing Ammonium Acetate and Methanol taken in the ratio 35:65 was pumped through column at a flow rate of 1.6 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 256.0 nm. Retention time of MET and NAT were found to be 2.804 min and 3.875 min. %RSD of the MET and NAT were and found to be 0.99 and 0.42 respectively. The method is linear over a concentration range of 10 to 50 µg/ml for MET and 60 to 300 µg/ml for NAT. The method was accurate with a %Recovery of98.6 to 99.7% and 99.87 to 100.2 % for MET and NAT respectively. The method developed is robust after making deliberate changes in flowrate, change in mobile are composition with a % RSD less than 2.Limit of Detection was found to be 0.015,&0.396µg/ml for MET and NAT respectively. Limit of Quantification was found to be 0.045,&1.18µg/ml for MET and NAT. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be utilized in regular analysis of dosage forms containing MET and NAT.

Keywords: Metformin Hydrochloride (MET),Nateglinide (NAT),Stability indicating studies, RP-HPLC.
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1. INTRODUCTION

Metformin Hydrochlorideis chemically named as 1-carbamimidamido-N,N-dimethylmethanimidamide. Metformin's mechanisms of action are unique from other classes of oral antihyperglycemic drugs. Metformin decreases blood glucose levels by decreasing hepatic glucose production (also called gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization [1]. It is well established that metformin inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through this mechanism[2, 3].

Nateglinide is chemically named as(2R)-3-phenyl-2-{[(1r,4r)-4-(propan-2-yl)cyclohexyl]formamido} propanoic acidis an oral antidiabetic agent used in the management of Type 2 diabetes mellitus [also known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes][4].

It is official in Indian Pharmacopoeia [5], British Pharmacopoeia [6], European Pharmacopoeia [7] and United States Pharmacopeia[8]. A literature survey revealed few Spectrophotometry, HPLC and UPLC methods for simultaneous estimation of individual and combined methods of MET and NAT in pharmaceutical formulation[9-12]. Pharmaceutical validations among these methods undergo the world 'Validation' means 'Assessment' of validity or action of providing effectiveness [13, 14] and validation as per ICH guidelines [15].

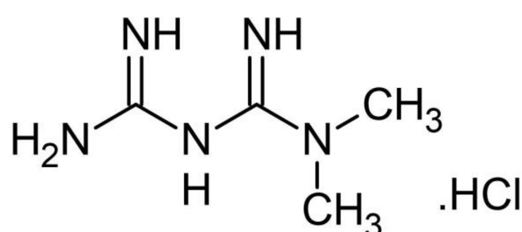

Fig 1: Structure of Metformin Hydrochloride (MET)

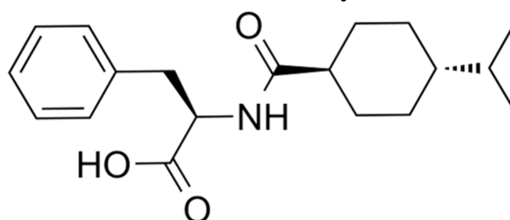

Fig.2: Structure of Nateglinide (NAT)

2. MATERIALS AND METHODS

MET and NATpure drugs (API) received from KP Labs.Combined form of MET and NAT tablets (IPCA Laboratories Ltd). Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogenortho phosphate buffer, perchloric acid Ortho-phosphoric acid. All the above chemicals and solvents are from Merck.

1. Solutions:

1.1. Preparation of Standard solutions:

10 mg of MET and 10mg of NAT were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to

dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution)

1.2. Samples Preparation

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 100 mg of active ingredient present in MET and NAT was transferred into a 100 ml clean dry volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 3 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as per with standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system.

1.3. Cc standards;

Calibration curve standards were prepared by pipetting suitable aliquots from stock solution into separate 10 ml volumetric flasks and the volume was made up to the mark with diluent to obtain the CC standards in the range of 10 - 50 µg/ml and 60 - 300 µg/ml concentrations for MET and NAT respectively.

2. Diluent: Mobile phase is used as diluent.

2.1. Chromatographic conditions:

The new HPLC method for estimation of MET and NAT was developed and validated using Symmetry C_{18} (4.6 x 150mm, 5µm).65 volumes of HPLC grade Methanol and 35 volumes of 0.01N Ammonium Acetate buffer adjusted to pH 3.0 (65: 35% v/v) as mobile phase. Separation was achieved through isocratic elution mode at 1.0 mL/min flow rate.

2.2. System suitability:

The system suitability parameters were determined by preparing standard solutions of MET (30µg/ml) and NAT (180µg/ml) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

2.3. Method validation

The method validation was performed in accordance with ICH guidelines

2.3.1. Linearity: Prepared standard dilutions of MET (10-50µg/ml) and NAT (60-300µg/ml) were injected and chromatogram was recorded in duplicate. A calibration curve was constructed by taking concentration on X- axis and average peak area on Y- axis.

2.3.2. Accuracy: Accuracy was determined by the recovery studies of the analyte. It is determined by standard addition method where the test solution of known quantity is spiked with standard solutions at three levels i.e., 50%, 100% & 150% in triplicate. Mean percentage recoveries at all the levels were calculated.

2.3.3. Precision: Precision of the method was established at two levels i.e., Repeatability and Intermediate precision. Repeatability was performed by injecting the working standard solution. Sixinjections were given from the same standard solution and the peak areas were obtained. Average area, standard deviation and % RSD were calculated for two drugs. Intermediate precision was established by taking multiple sampling from a sample stock solution prepared by two different analysts. Six working sample solutions of same concentrations were injected by Analyst-1 and Analyst- 2. Average peak area, standard deviation and pooled % RSD were calculated for two drugs

2.3.4. Robustness:Small deliberatechanges in method like Flow rate and mobile phase ratio, are made. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, was maintained and samples were injected in sextet. System suitability parameters are evaluated by making the deliberate changes.

2.3.5. Specificity:The specificity was studied by establishing the interference of placebo with the drug. A sample of placebo was injected into the HPLC system as per the test procedure. Chromatogram of placebo should not show any peak at the retention time of analyte peak.

3. RESULTS AND DISCUSSION

1. Assay of formulation:

Assay of the formulation is performed as per the given procedure. This was done in triplicate. The amount of drug present in the formulation was calculated from standard graph. The % assay of MET and NAT obtained was 99.48 and 98.03% respectively. Representative chromatograms for standard and test were given in figures 3 &4. Results were given in the table 1.

2. System suitability:

System suitability parameters were determined according to ICH guidelines. Plate count was more than 2000, tailing factor was less than 2 and resolution was more than 2. All the system suitable parameters were passed and were within the limits. The results showing system suitability parameters were given in table no. 2

3. Validation:

3.1. Linearity:

The linearity was determined at six concentration in the range of 10 - 50 µg/ ml for MET and 60 - 300 µg/ ml for NAT. The Peak areas against concentration were plotted and the calibration curve was constructed. The calibration curves were illustrated in figures 5 & 6. The Correlation coefficient (r^2) was greater than 0.99 within the concentration range for both the drugs. The results for linearity were given in the table 3.

3.2. Accuracy:

Accuracy of the method was established at three levels of concentrations by standard addition method. Triplicate injections were given at each level of accuracy and percentage recoveries were calculated. The mean % Recovery was obtained was 100.14 % and 100.07 % for MET and NAT respectively. The results for accuracy were given in the table 4.

3.3. Precision:

The precision of the method was studied by considering repeatability and intermediate precision. Repeatability was studied by taking six replicate injections from same homogenous standard solution and peak areas were determined. Average area, standard deviation and % RSD were calculated for two drugs. Intermediate precision was studied by determining analyst to analyst variation by preparing six test solutions of same concentration and injected once from each solution and peak areas were determined by analysts 1 & 2. The % RSD of MET for repeatability and intermediate precision was found to be 0.99 and 0.83, for NAT it was found to be 0.42 and 0.71. It passes repeatability and intermediate precision. The results for precision were given in the table 5

3.4. Robustness:

Robustness of the method was studied by making deliberate changes in flow rate, and mobile phase ratio. After making each change in the conditions, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level. System suitability parameters were not much affected and all the

parameters were passed. % RSD was within the limit. Results were given in the table 6.

3.5. Specificity:

The Chromatograms of Standard and Sample are identical with nearly same Retention time. No interference due to Placebo and Sample at the retention time of analyte which shows that the method was specific.

Table No. 1: Assay Data

S. No.	Peak area of MET	Peak area of NAT
1	386989	2342352
2	387093	2335575
3	401018	2444315
Avg	387041	2338963.5
Regression equation	y = 5633 x + 16910.	y = 5404.x + 1432.1
% Assay	98.48%	98.03%

Table No. 2: System Suitability parameters forMET and NAT

SAMPLE	R_t	Peak Area	USP plate count	USP Tailing
MET	2.799	386989	2598.1	1.6
NAT	3.861	2444315	4137	1.5

Table No. 3: Linearity data of MET and NAT

MAT		NAT	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
10 ppm	228407	**60 ppm**	1616125
20 ppm	276978	**120 ppm**	1868367
30 ppm	339892	**180 ppm**	2234843
40 ppm	393459	**240 ppm**	2571642
50 ppm	451862	**300 ppm**	2885708
R^2	y = 5633.x + 16910	**R^2**	y = 5404.x + 1432.1
	0.998		0.997

Table No. 5: Accuracy data of MET and NAT

% Level N=3	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery
MET			
50 %	15	14.6	98.6
100%	30	29.91	99.7

150 %	45	44.86	99.6
NAT			
50 %	30	30.17	100.56
100%	60	60.17	100.29
150 %	90	89.05	98.95

Table No. 6: Precision data of MET and NAT

	MET		**NAT**	
Injection No	**Peak Area**	**R_t**	**Peak Area**	**R_t**
1	368013	2.808	2321302	3.880
2	372552	2.808	2308016	3.880
3	367873	2.808	2326058	3.880
4	375555	2.808	2334897	3.880
5	374843	2.808	2326143	3.880
Avg	371767		2323283	
SD	3663.5		9845.8	
% RSD	0.99		0.42	

Table No. 7: Robustness data of MET and NAT

S. No.	**Condition**	**% RSD of MET**	**%RSD of NAT**
1	Flow rate (-) 0.9ml/min	0.3	0.8
2	Flow rate (+) 1.1ml/min	1.1	0.7
3	Mobile phase (-) 60B:40A	0.6	0.8
4	Mobile phase (+) 50B:50A	0.5	0.9

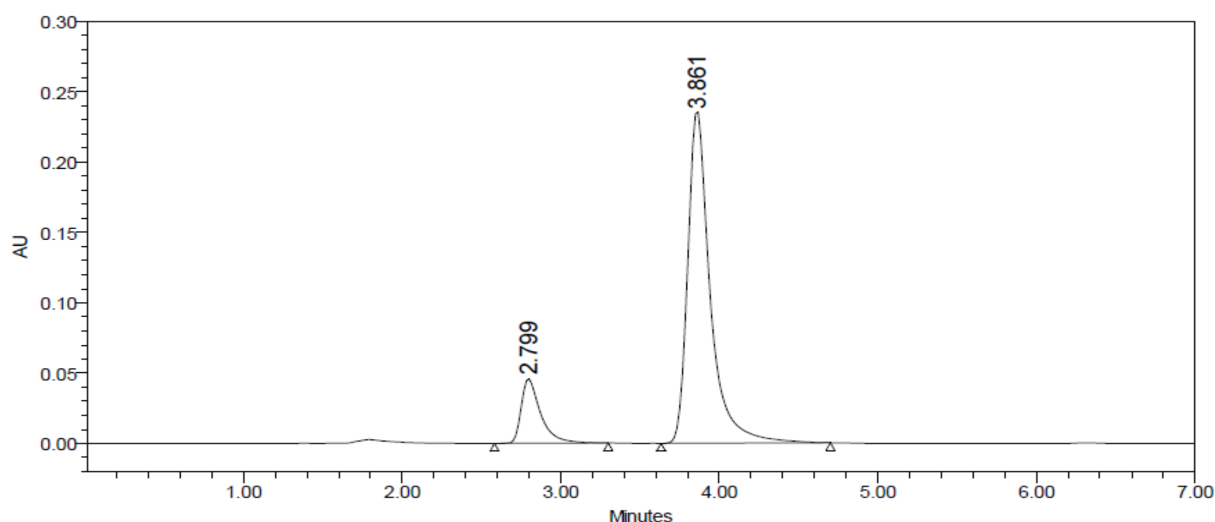

Fig. 3: Representative Chromatogram of working standard solution

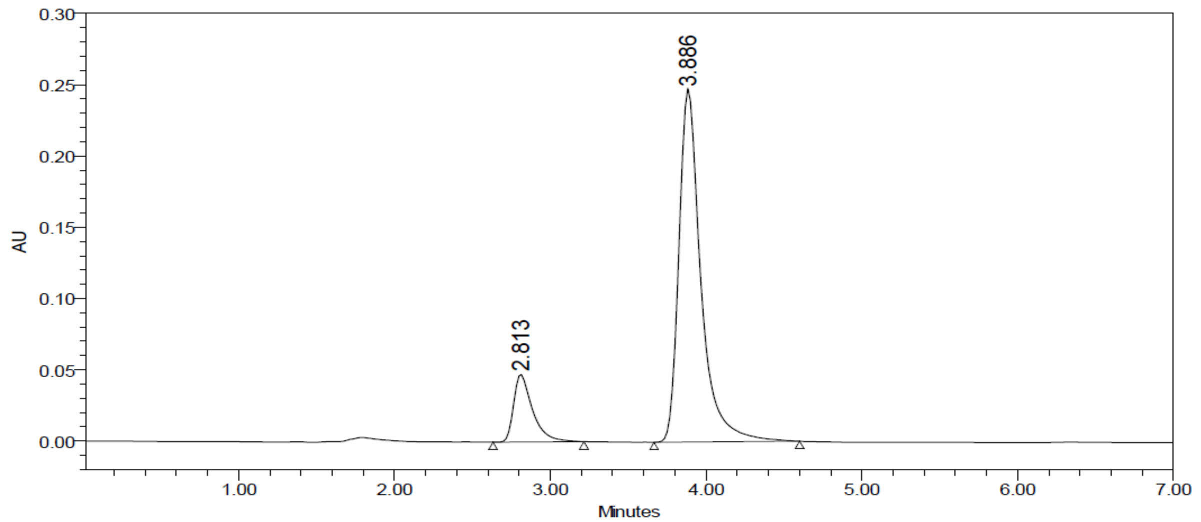

Fig. 4: Representative Chromatogram of working sample solution

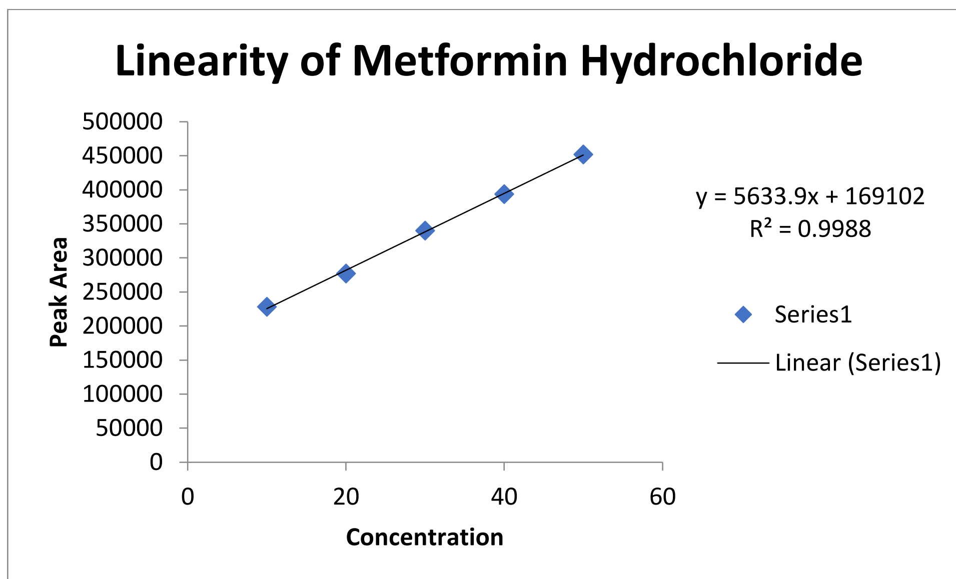

Fig. 5: Calibration Curve of MET

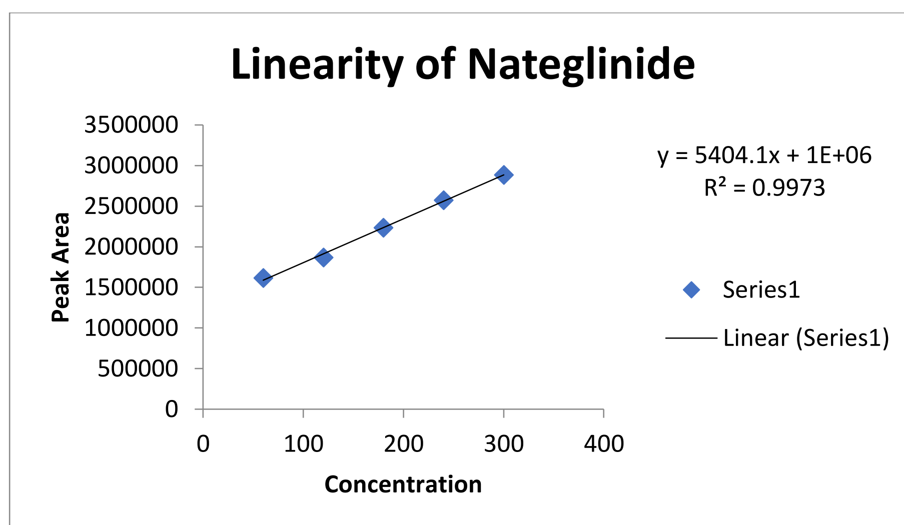

Fig. 6: Calibration Curve of NAT

4. CONCLUSION

Reverse Phase High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of MET and NAT was done by RP-HPLC. The Ammonium acetate buffer was pH 3 and the mobile phase was optimized with consists of Methanol: Ammonium acetate buffer mixed in the ratio of 65:35 % v/v. A Symmetry C_{18}column C_{18} (4.6 x 150mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase.. The solutions were chromatography at a constant flow rate of 1.2 ml/min. the linearity range of MET and NAT were found to be from 10-50 µg/ml of MET and 60-300µg/ml of NAT. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-101% of MET and NAT. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. REFERENCES

1. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/021748s025lbl.pdf.
2. Rena G, Hardie DG, Pearson ER: The mechanisms of action of metformin. Diabetologia. 2017 Sep;60(9):1577-1585. doi: 10.1007/s00125-017-4342-z. Link: https://go.drugbank.com/articles/A36534.
3. Rena G, Pearson ER, Sakamoto K: Molecular mechanism of action of metformin: old or new insights? Diabetologia. 2013 Sep;56(9):1898-906. doi: 10.1007/s00125-013-2991-0. Link: https://go.drugbank.com/articles/A36557.
4. https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021204s014lbl.pdf.
5. Indian Pharmacopoeia, Government of India, Ghaziabad. The Indian Pharmacopoeia Commission. 2007;2:1358.
6. British Pharmacopoeia, Her. Majesty's Stationary Office. London. UK. 2009;1 and 2:3813.
7. European Pharmacopoeia. Council of Europe, France. 3rd Edition. 1997; 55.
8. The United States Pharmacopoeia. US Pharmacopoeial convention. Inc. Rockville, MD. 31st Revision. 2008;2640.
9. Madhukar. A et. al., Simple and Sensitive Analytical Method Development and Validation of Metformin Hydrochloride by RP-HPLC. Int J Pharm PharmSci, 2011;3(3):117-120.
10. Vijay Kumar et al. A Novel Simultaneous Stability-Indicating UPLC Assay Method for Metformin Hydrochloride and Canagliflozin in Oral solid dosage form.Int J Adv Pharm Biotech, 2018;4(1):10-22. DOI: doi.org/10.38111/ijapb.20180401002.
11. GadapaNirupa, Upendra M. Tripathi. RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms. J Chem, 2013, Article ID 726235, 8 pages, 2013. DOI: https://doi.org/10.1155/2013/726235.

12. Chengalva et al, Development and Validation of RP-HPLC Method for Metformin hydrochloride and Nateglinide in Bulk and Combined dosage form. Int J Pharm PharmSci, 2016;8(4):267-271.
13. Sharma S.K. Validation of pharmaceutical products and process. The Eastern Pharmacist. July 2001;21-23.
14. Chowdary K.P.K., Himabindu G. Validation of analytical methods. Eastern Pharmacist. May 1999;39-41.
15. Validation of analytical procedures/methodology. ICH harmonized triplicate guideline. 1996;1-8.